Reference: *Biol. Bull.* **164**: 176–188. (April, 1983)

NEURAL CONTROL OF METAMORPHOSIS IN *DENDRASTER EXCENTRICUS*

ROBERT D. BURKE

Department of Biology, University of Victoria, Victoria, B. C., Canada V8W 2Y2, and Bamfield Marine Station, Bamfield, B. C., Canada V0R 1B0

Abstract

Glyoxilic acid induced fluorescence histochemistry and ultrastructural observations indicate that the larval nervous system includes an apical region of neuropile on the oral hood between the anterolateral arms and a ganglion comprised of nerve cell bodies and neuropile on the lower lip of the larval mouth. Electrical stimulation induced metamorphosis more frequently and with a lower mean threshold when a suction electrode was attached to either of these two nerve centers. When the entire oral hood, including oral ganglion and apical neuropile was excised, it and the posterior regions of the larva metamorphose spontaneously. Neither the excised pre-oral hood nor the remaining portions containing the oral ganglion and the adult rudiment metamorphose until exposed to the chemical cue that naturally induces metamorphosis. Excised larval arms do not respond to the natural cue. 10^{-5} *M* dopamine induced metamorphosis in a small proportion of larvae, and 10^{-5} *M* L-dopa and dopamine induced metamorphic responses in excised larval arms. It is proposed that the apical neuropile and oral ganglion are nerve centers that mediate between the perception of the natural cue and control the initiation of metamorphosis.

Introduction

The larvae of numerous species of marine invertebrates settle and metamorphose in response to certain factors associated with preferred adult habitats (Meadows and Campbell, 1972; Crisp, 1974; Chia and Rice, 1978). The paradigm is that larvae grow and develop to a state in which they are competent to metamorphose, and remain so until they encounter stimuli that induce metamorphosis (Crisp, 1974, 1976; Chia, 1978). Metamorphosis in most marine invertebrates is a relatively rapid sequence of developmental events that alters the form and function of the organism (Scheltema, 1974; Cloney, 1978). It has been surmised that these developmental events are held in abeyance by some endogenous physiological mechanism, though the nature of this control remains to be determined. It has been suggested that an endocrine system controls metamorphosis in decapod crustaceans in a manner similar to that of insects (Passano, 1961) but the evidence remains equivocal (Little, 1969). In other groups, neural control has been suggested (Hadfield, 1978; Morse *et al.*, 1979; Marsden and Anderson, 1981; Cloney, 1982).

Larval development and metamorphosis of several species of echinoids is typical of this model in many ways. Larvae undergo a predictable sequence of developmental changes during which a rudiment of the adult form develops on one side of the larva (MacBride, 1903, 1914, 1918). Competent larvae of several species metamorphose in response to cues from the adult environment (Caldwell, 1972;

Received 3 September 1982; accepted 25 January 1983.

Cameron and Hinegardner, 1974; Highsmith, 1982). Metamorphosis is a rapid sequence of irreversible developmental events in which the adult rudiment is everted and the larval body retracted and resorbed (Cameron and Hinegardner, 1978; Chia and Burke, 1978; Burke, 1982). This report presents evidence indicating that the larval nervous system of the pacific sand dollar, *Dendraster excentricus* controls the initiation of metamorphosis.

Materials and Methods

Larval culture

Larvae were reared from fertilized eggs using the standard procedures outlined by Strathmann (1968) and Hinegardner (1969). Adults were collected subtidally from Breaker Beach, Bamfield, British Columbia, Canada. Spawning was induced by intracoelomic injection of 0.55 *M* KCl. Larvae were kept in 2 l battery jars supplied with a slow stream of air bubbles for agitation. Water was changed on alternate days and *Dunaliella salina* was supplied at a concentration of about 10^4 cells ml^{-1} for food.

Microscopy

Catecholamine containing cells were visualized by the glyoxilic acid induced fluorescence technique described by Sharpe and Atkinson (1980). Larvae were placed in a solution containing 2% glyoxilic acid and 0.1 *M* phosphate buffer (pH 7.0, 4°C). After 3 to 5 min, larvae were placed on a glass slide and dried with a hair drier. The slides were heated to 100°C on a hot plate for 5 min before being mounted in liquid paraffin under a thin, glass cover slip. Specimens were examined immediately with a Zeiss Universal microscope fitted with UV epifluorescence equipment. An excitation filter with a peak transmittance of 364 nm was used on conjunction with a chromatic beam splitter (FT420) and a barrier filter (LP 418) reflecting light below 418 nm.

Larvae were fixed for electron microscopy in a solution of 2.5% glutaraldehyde in 0.12 *M* phosphate buffer, 0.14 *M* NaCl, and 0.2% tannic acid (Simionescu and Simionescu, 1976). Specimens were post fixed for 1 h at room temperature in 2% osmium tetroxide in 1.25% $NaHCO_3$. After dehydration by alcohol exchange, specimens for transmission electron microscopy (TEM) were infiltrated and embedded in Polybed (Polysciences Inc.). Sections were cut on a diamond knife and mounted on parlodion coated copper grids and stained with 50% ethanol saturated with uranyl acetate, followed by lead hydroxide chelated with sodium citrate. Observations and micrographs were made using a Philips EM300 electron microscope.

Electrical stimulation

Competent larvae were electrically stimulated after attachment to a fine tipped, polyethylene suction electrode (tip diameter 25–50 μm). Stimuli were from a Grass S44 square wave stimulator used in conjunction with a Grass SIU5 stimulus isolation unit. Threshold levels were determined by increasing the voltage output by 5 volt increments between 5 and 50 volts and by 25 volt increments to 150 volts. Stimuli were repetitive pulses of 5 ms duration delivered at a rate of 5 pulses per s for a total of 10 s. Preparations were allowed to stabilize for one min between treatments, and temperatures in the bath were maintained between 10° and 15°C.

Excisions and chemical stimulation

Portions of competent larvae were excised using tungsten needles prepared by etching in hot sodium nitrite. The reactions of excised parts and whole larvae to various chemicals was tested. Neurotransmitter and neuroinhibitor substances were initially prepared as 10^{-3} *M* stock solutions that were diluted immediately before use in 0.45 μm filtered sea water. A crude preparation of the natural cue to metamorphosis was made by stirring sand from an aquarium containing adult sand dollars in an equal volume of sea water for 5 min and then filtering the water. The crude preparation was tested for activity using 10 to 20 competent larvae. Only preparations that would induce greater than 90% metamorphosis within 5 min were used.

Results

Larval nervous system

Glyoxilic acid induces a bright blue-white fluorescence of what are apparently nerves in the pluteus of *D. excentricus* (Fig. 1). These nerves are associated predominantly with the ciliary bands surrounding the arms and the larval mouth. Diffuse fluorescence associated with the adult rudiment and bright red fluorescence in the gut were considered artifactual. Fluorescence associated with ciliary bands appears as narrow tracts, 1–5 μm wide at the base of the ciliary epithelium (Figs. 1b, c, f). The tracts consist of axons less than 0.5 μm thick. The number of axons is greatest in regions of ciliary band between the larval arms and least within the arms themselves. Although there were some bright spots of fluorescence, 1–2 μm in diameter within the tracts, there were no obvious cell bodies.

A prominent oral ganglion is located within the adoral ciliary band (Fig. 1e). Forty to fifty brightly fluorescing cell bodies are dispersed along the lower lip of the larval mouth. These cells appear flask shaped, are 10 μm wide basally, and form a narrow neck, 2.5 μm wide, that extends apically up to 20 μm. The basal portions of the cells are associated with a neuropile from which numerous dendritic projections extend. The adoral ciliary band is organized in a manner similar to the ciliary bands of *Strongylocentrotus purpuratus* described by Burke (1978). Spindle shaped ciliated cells surround an accumulation of axons located basally in a central position. In the lower lip, the axons form a neuropile that is up to 15 μm in diameter and is a major portion of the band. The axons that make up the neuropile range from 0.3 μm to 0.6 μm in diameter (Figs. 2a, c). They contain mitochondria, microtubules, and numerous vesicles, 60–75 nm in diameter which frequently contain an electron dense core (Figs. 2c, d). Interspersed with the axons are verrucosities, 1.5 to 2 μm in diameter containing a dispersed flocculent material. The axons also contain vesicles that are frequently located adjacent to the plasmalemma (Figs. 2a, d). Occasionally, cells within the ciliated epithelium contributed a process to the axonal tract (Fig. 2b). These cells typically have a basally located nucleus, 5 μm in diameter, and a narrow neck extending toward the surface of the epithelium. The cytoplasm of these cells contains microtubules, clumped ribosomal material, and numerous vesicles up to 1 μm in diameter, containing course granular or flocculent material.

A region of neuropile, 80 μm by 50 μm is located between the anterolateral arms of the apical surface of the oral hood (Fig. 1d). Numerous axons form a swollen commissure between the axonal tracts of the ciliary bands. An overall diffuse brightness is associated with the neuropile, and some axons fluoresce more brightly than others, but no cell bodies were apparent. Ultrastructurally the apical neuropile is

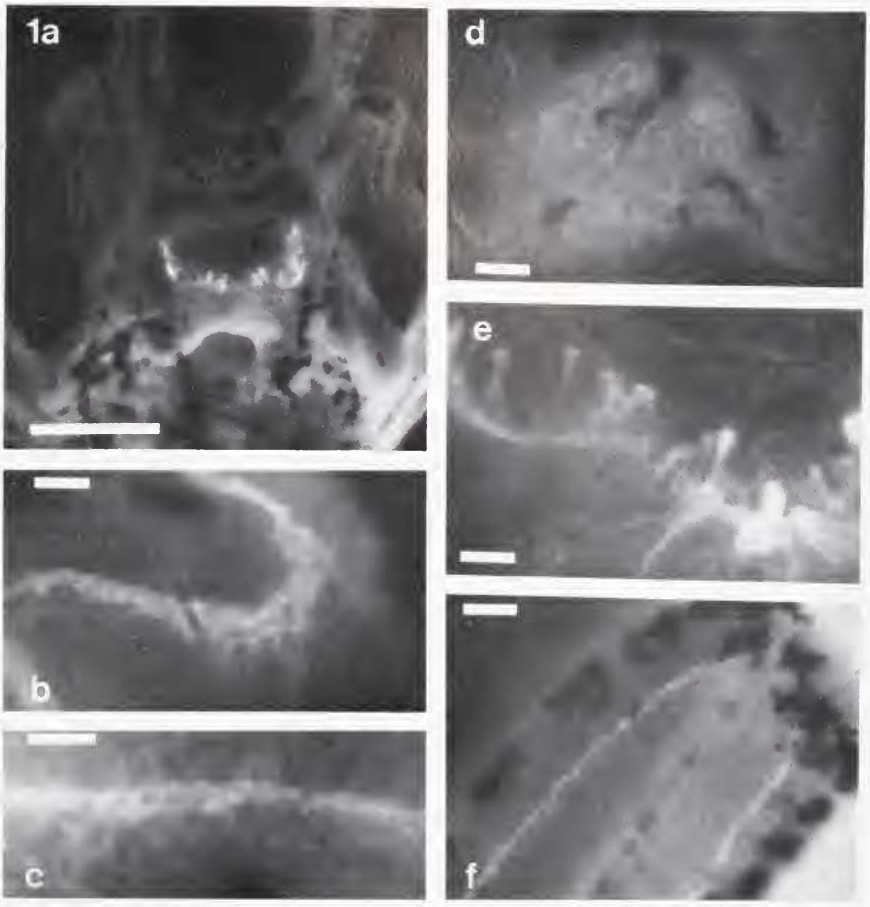


FIGURE 1 a. Competent larva of *Dendraster excentricus* prepared for glyoxilic acid induced fluorescence of catecholamines. Bar = 100 μm. b. Detail of a region of ciliary band between post-oral and posterodorsal arms of competent larva. Bar = 10 μm. c. Portion of pre-oral transverse ciliary band. Bar = 10 μm. d. Apical neuropile of competent larva, dark regions are pigment cells that overlie the neuropile. Bar = 10 μm. e. Detail of the oral ganglion. Bar = 10 μm. f. Tip of post-oral arm. Bar = 10 μm.

a plexus of axons and verrucosities similar to the axonal tract of the oral ganglion described above (Fig. 3). It is up to 20 μm thick and is covered by a 10 μm thick epithelium.

Electrical stimulation

Direct electrical stimulation induced immediate metamorphosis when a suction electrode was attached to either the oral ganglion or the apical neuropile (Table I, Fig. 4). Metamorphosis could also be induced when the electrode was attached to the oral surface of the adult rudiment or the ciliary band on the dorsal surface between the posterodorsal arms, but these locations were successful in less than half of the preparations attempted, and mean thresholds were higher than those needed for the oral ganglion or the preoral plexus. Stimulation with the electrode attached to the epidermis at the posterior end never induced metamorphosis.

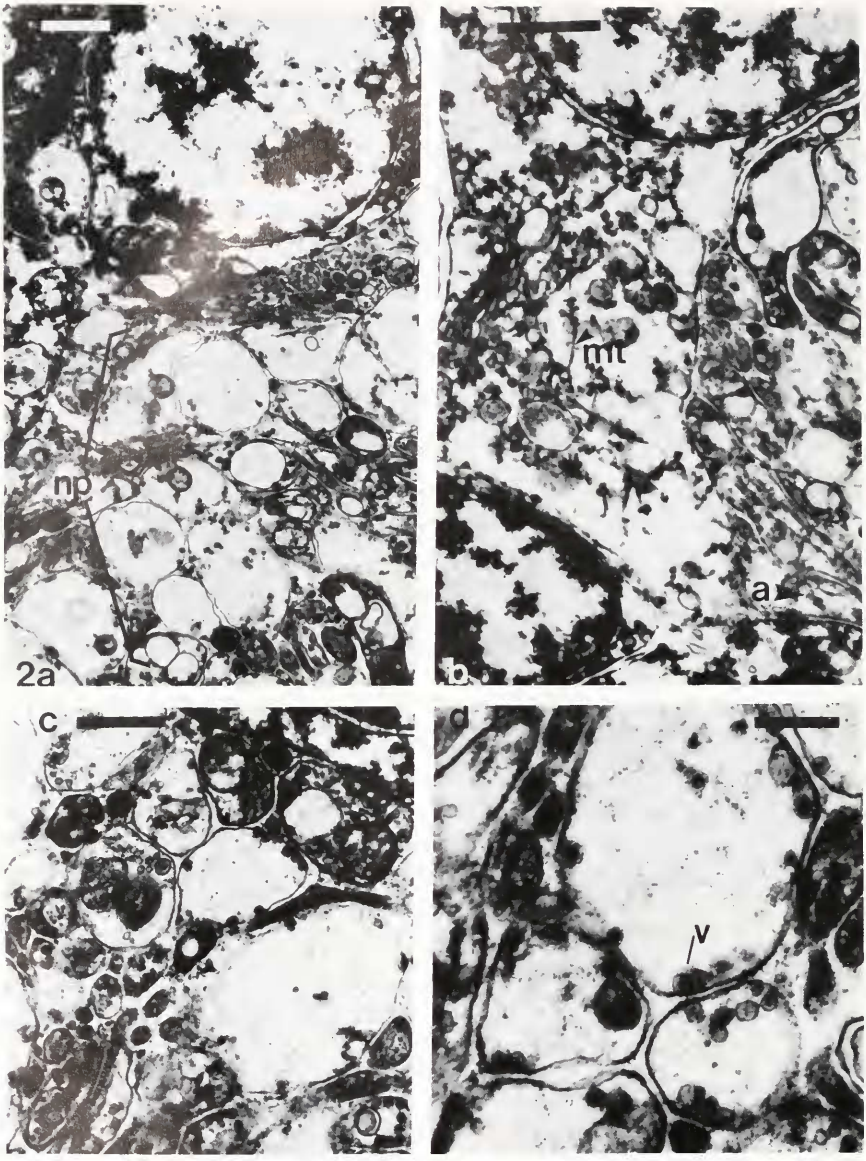


FIGURE 2 a. Transverse section through the oral ganglion of competent larva of *Dendraster excentricus*. Bar = 1 μm. b. Section through cell of epithelium overlying the neuropile which appears to contribute an axonal process (a) to the neuropile. Bar = 0.5 μm. c. Detail of neuropile from transverse section through it. Bar = 0.5 μm. d. Varrucosities of axons located within the neuropile region of the oral ganglion. Bar = 0.25 μm. a, axonal process; mt, microtubules; np, neuropile; v, vesicles.

Typically, when the electrode was attached to either the apical neuropile or the oral ganglion, stimuli of 1 to 5 volts evoked ciliary reversals and twitch responses from the larvae. Once the threshold was reached, the adult rudiment was activated, as indicated by movements of the spines and tube feet, and the entire oral hood flexed ventrally (Fig. 4b). Eversion of the adult rudiment followed within 1 to 2

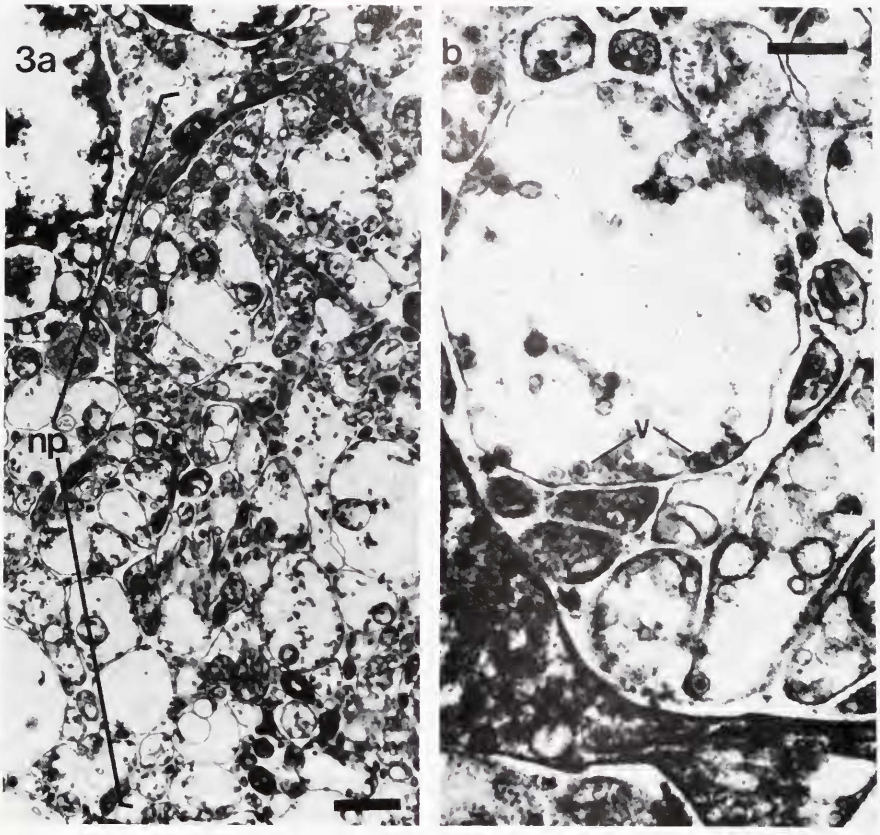


FIGURE 3 a. Transverse section through apical neuropile of competent larva of *Dendraster excentricus*. Bar = 1 μm. b. Detail of neuropile showing axonal profiles and varrucosites. Bar = 0.25 μm. np, neuropile; v, vesicles.

min, and the remainder of metamorphosis proceeded in typical sequence. Juveniles that were induced to metamorphose by electrical stimulation appeared normal and lived indefinitely.

When the electrode was attached to the ciliary band on the dorsal surface of the larva between the posteriodorsal arms, the oral surface of the adult rudiment, or

TABLE I

Summary of experiments correlating position of the electrode with the threshold of stimuli that will induce immediate metamorphosis of competent larvae of Dendraster excentricus

	Electrode location				
	Oral ganglion	Apical neuropile	Posterior end	Ciliary band	Oral surface of rudiment
Number metamorphosed	7/8	7/8	0/5	3/7	3/7
Mean threshold (Volts)	18.6 ± 6.3	22.9 ± 13.8	*	83.3 ± 28.9	83.3 ± 57.7

* Metamorphosis was never induced with electrode located here regardless of the intensity of stimulation.

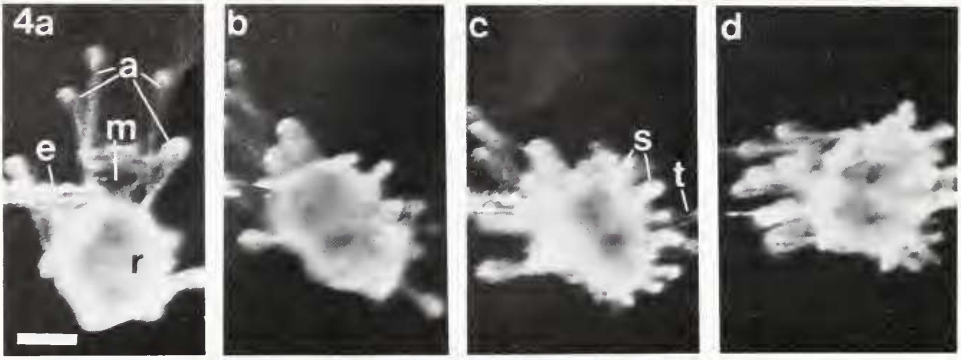


FIGURE 4. Competent larva of *Dendraster excentricus* attached to a fine-tipped, suction electrode in region adjacent to the oral ganglion. a. Prior to stimulation. b. 1 min after stimulation, pre-oral region has flexed anteriorly and the oral rudiment has activated and begun to evert. c. 4 min after stimulation, rudiment is fully everted and epithelium of larval arms has begun to retract. d. 10 min after stimulation, larva is no longer attached to the electrode and retraction of arm epithelium has been about half completed. Bar = 100 μm. a, arms; e, electrode; m, larval mouth; r, adult rudiment; s, spines on adult rudiment; t, tube feet.

the posterior end of the larva, responses such as ciliary reversals or twitches of larval muscles were not evoked until stimuli were 15 to 20 volts. If the threshold for metamorphosis was reached, larvae responded immediately in the same manner as described above. If the threshold was not reached, and the electrode was repositioned to the apical neuropile or the oral ganglion, metamorphosis could not be induced at any stimulation potential.

Excisions

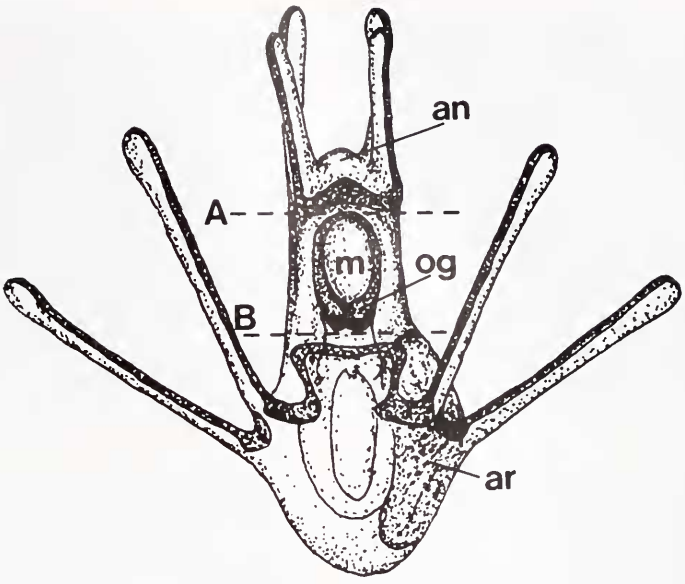
Competent larvae can be dissected using fine tungsten needles. When the entire oral hood, including both the oral ganglion and the apical neuropile, was excised both halves underwent their characteristic reactions of metamorphosis (Table II, Figs. 5, 6). The adult rudiment was activated and everted within 15 min. A normal

TABLE II

Summary of experiments examining the effects of excisions of certain parts of the larval body on metamorphosis of competent larvae of Dendraster excentricus

Treatment	Number that underwent metamorphic Reaction*	
	Excised part	Part containing adult rudiment
Oral hood excised (includes oral ganglion and apical neuropile)	12/15	11/15
Preoral hood excised (includes only apical neuropile)	3/15	2/15
Preoral hood excised and subsequently treated with crude preparation of cue for metamorphosis (15 min)	11/12	10/12
Larval arms excised	0/20	0/20
Larval arms excised and subsequently treated with crude preparation of cue for metamorphosis (1 h)	0/20	19/20

* Metamorphic reactions described in text.



5

FIGURE 5. Sketch of *Dendraster excentricus* larva showing levels at which cuts were made to excise preoral hood (A) and oral hood (B). an, apical neuropile; ar, adult rudiment; m, mouth; og, oral ganglion.

looking juvenile resulted. The oral hood's response was the sequence of contraction and histolysis that the larval epidermis typically undergoes during metamorphosis in intact larvae (Cameron and Hinegardner, 1978; Chia and Burke, 1978) (Fig. 6). In larvae in which only the pre-oral hood was removed so that the portion containing the adult rudiment also contained the tissues of the larval mouth and the oral ganglion, neither portion spontaneously metamorphosed within 1 h (Table II, Figs. 5, 6). When these two portions of the larva were exposed to a crude preparation of the cue that induces metamorphosis in intact larvae, both portions metamorphosed within 15 min. When individual larval arms were removed from larvae, the cilia continued to beat causing the arms to swim for at least 24 h (Table II). Arms treated with the crude preparation of the natural cue to metamorphosis also swam indefinitely showing no signs of the contraction-histolysis response of metamorphosis.

Neurotransmitters and neuroinhibitors

When larvae were put into SW containing neurotransmitter substances, metamorphosis was induced by dopamine, but with relatively low incidence (Table III). Excised larval arms responded to L-dopa and dopamine in a more consistent manner by undergoing the contraction-histolysis sequence of metamorphosis (Table IV).

To test the effects of catecholamine neuroinhibitors, larvae were exposed to the natural cue for metamorphosis in the presence of an inhibitor. Only 10^{-4} *M* reserpine appeared to interfere with the induction of metamorphosis; 6/18 metamorphosed within one hour, whereas those treated with the cue alone (19/20) and those treated with 10^{-4} isoproterenol (20/20) appeared unaffected. Lower concentrations of either drug also appeared to have no effect. Neither drug, at any of the concentrations attempted, had any effect on the induction of metamorphosis by electrical stimulation.

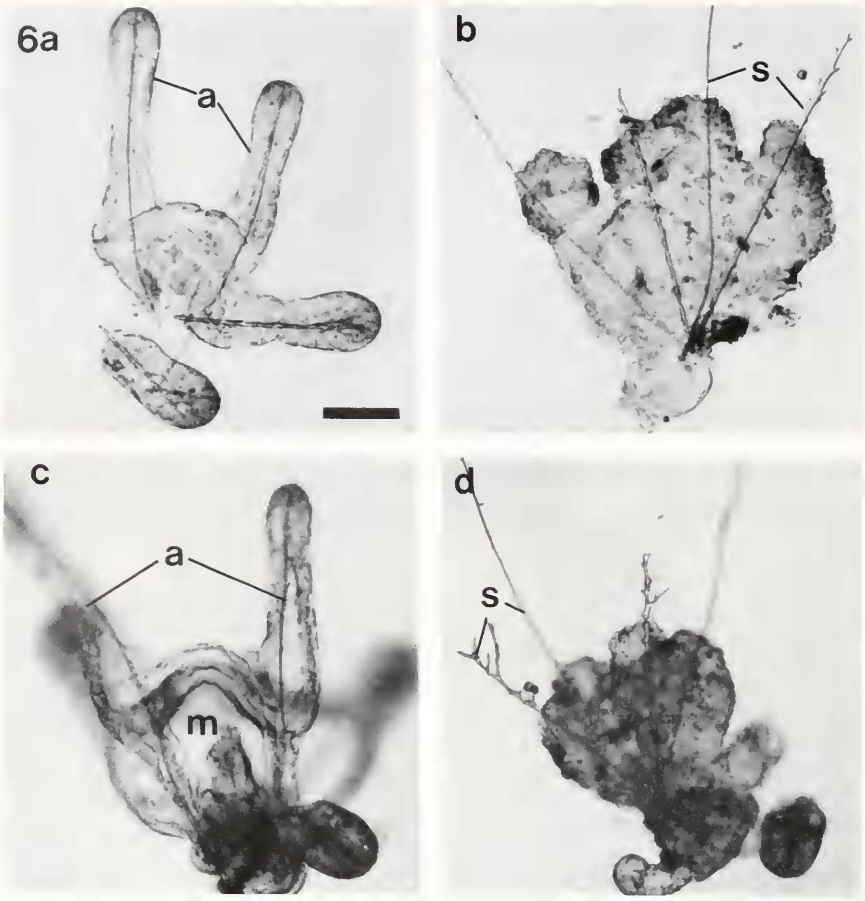


FIGURE 6. Excised pre-oral hood of competent larva of *Dendraster excentricus* after one h in sea water. b. Excised pre-oral hood after 30 min exposure to crude preparation of the cue for metamorphosis. c. Excised oral hood region. d. The same oral hood as is figured in c, after fifteen min in sea water. Bar = 100 μm. a, larval arms; m, larval mouth; s, larval skeleton.

DISCUSSION

Several structures in the echinopluteus have been suggested to be parts of a larval nervous system. MacBride (1914) identified the apical complex, a thickened epithelium on the oral hood, as a nervous structure. Mortensen (1920) suggested that the adoral ciliary band contains nerves. Gustafson (1969) and Ryberg (1977) propose that neuron-like cells spanning the blastocoel comprise a larval nervous system in *Psamnechinus miliaris*. Burke (1978) described a system of nerve cells and axonal tracts associated with the ciliary bands and esophageal muscles in *Strongylocentrotus purpuratus*. The histochemical evidence presented here supports the idea that the nervous system consists of tracts of axons in the ciliary bands and corroborates, in part, the histochemical evidence presented by Ryberg (1974) for localization of biogenic amines in the ciliary bands and parts of the larval gut.

The structure of both apical and adoral neuropiles is similar to that of the radial nerves and nerve rings of adult echinoderms (Pentreath and Cobb, 1972). All of

TABLE III

Summary of experiments testing the effects of neurotransmitter substances on competent larvae of Dendraster excentricus

Neurotransmitter	Number metamorphosed (1 h)		
	10^{-3} *M*	10^{-4} *M*	10^{-5} *M*
Acetylcholine Chloride	—	—	—
5-Hydroxytryptamine	—	—	—
γ-Amino-n-butyric Acid	—	—	—
Epinephrine	—	—	—
Noradrenaline	—	—	—
3-Hydroxytyramine (Dopamine)	5/20	1/20	5/20
L-β-3,4-Dihydroxyphenylalanine (L-DOPA)	—	—	—

these structures consist of tracts of relatively densely packed nerve fibers aligned longitudinally. Nerve cell bodies are only rarely encountered within these regions (Cobb, 1970; Pentreath and Cobb, 1972). The radial nerve cords and nerve rings of adult echinoderms have been ascribed the role of a central nervous system (Smith, 1965; Pentreath and Cobb, 1972). Similar accumulations of axons may function as coordination and integration centers within the larval nervous system. The neuropile associated with the lower lip of larvae is surrounded by nerve cell bodies, so the structure has been termed a ganglion. The connection of these cell bodies with the external surface suggests that they may play a sensory role.

Experiments in which the position of the stimulating electrode was varied show that when the stimulus is delivered to regions associated with either the apical neuropile or the oral ganglion, metamorphosis is more reliably induced and the mean threshold of stimulation is less than at other locations. It is proposed that metamorphosis results because of direct stimulation of the nerves in these regions. Elements of the nervous system are associated with all of the other sites of stimulation, but the apical neuropile and oral ganglion appear to be most effective in inducing metamorphosis. Cameron and Hinegardner (1974) reported that *Arbacia punctulata* larvae metamorphosed in response to electrical stimulation of 150 V delivered in 1 ms pulses. They did not note any specific locations for the placement of the electrode, but as results reported here indicate, electrode placement is not critical with high voltage stimuli. Higher voltage stimuli may cause tissue damage,

TABLE IV

Summary of experiments testing the effects of neurotransmitter substances on excised larval arms

Neurotransmitter	Number undergoing metamorphic reaction (1 h)		
	10^{-4} *M*	10^{-5} *M*	10^{-6} *M*
Acetylcholine Chloride	—	—	—
5-Hydroxytryptamine	—	—	—
γ-Amino-n-butyric Acid	—	—	—
Epinephrine	—	—	—
Noradrenaline	—	—	—
3-Hydroxytyramine (Dopamine)	4/6	6/6	—
L-β-3,4-Dihydroxyphenylalanine (L-DOPA)	2/6	6/6	—

as larvae for which a threshold was never reached did not metamorphose when the electrode was repositioned.

The excision experiments demonstrate interactions between tissues of the oral hood and the remainder of the larva containing the adult rudiment. Because both halves of larvae dissected below the larval mouth will spontaneously metamorphose, it is proposed that there is a mutual inhibitory control between these portions of the larva. Since excision of the pre-oral region does not induce spontaneous metamorphosis, it is deduced that inhibitory control is localized in the region of the larval mouth.

The excised pre-oral region of the larva and the remaining portion of the larva containing the oral regions and the adult rudiment will only undergo metamorphic reactions in the presence of the natural cue for metamorphosis. This suggests that both portions contain receptors for the cue and the necessary mechanisms for transmitting this stimulus to all the tissues involved in the metamorphic response. Because tissues of the larval arms are apparently unable to perceive the cue, or initiate the contraction and histolysis response, it is proposed that a stimulatory center is localized in the pre-oral region of the larva. A second stimulatory center would have to be located in the portion of the larva containing the oral regions and the adult rudiment.

Cameron and Hinegardner (1974) showed that larvae of *Arbacia punctulata* are induced to metamorphose by a combination of a soluble chemical cue and tactile stimulation of the primary podia of the adult rudiment. Burke (1980) has suggested that sensory receptors on the tips of the primary podia are involved in the perception of these cues in several species of regular echinoid. *D. excentricus* differs from *A. punctata* in that larvae of the former respond to a chemical cue alone, and the adult rudiment remains inactive prior to the initiation of metamorphosis. Although *D. excentricus* possess similar podial sensory receptors (Burke, unpublished observations), experiments reported here indicate that the receptors that receive the cue may be more dispersed. Although the excision experiments indicate that there is a stimulatory center located on the pre-oral hood and another in the regions posterior to it, it does not definitely indicate that these centers correspond to the locations of receptors. However, receptors must be located somewhere on the pre-oral hood.

Glyoxilic acid induced fluorescence has been suggested to be specific for dopaminergic nerve cells (Grace and Bunny, 1980; Sharpe and Atkinson, 1980), however Keenan and Koopowitz (1981) have presented evidence that glyoxilic acid will induce fluorescence of L-dopa, L-dopamine, L-tyrosine, and norepinephrine and emittence spectra for all these catecholamines are indistinguishable. The observation of vesicles with an electron dense core, similar to those observed in catecholamine containing nerves in adult echinoderms (Pentreath and Cobb, 1972), also supports the hypothesis that larval nerves contain catecholamines.

The inconsistent response of whole larvae to dopamine is difficult to interpret, though it does suggest dopamine may be involved in some stage of the induction of metamorphosis. The responses of isolated larval arms were more consistent, though relatively high doses for neurotransmitters were necessary to bring about a response. These observations are compatible with the hypothesis that either L-dopa, dopamine, or a derivative of them acts as a chemical messenger during the induction of metamorphosis. This endogenous chemical signal may act directly on the larval and adult tissues that respond during metamorphosis, or it may act indirectly by causing the release of additional substances that stimulate the tissues containing the effectors of metamorphosis. The fact that reserpine, a substance thought to deplete catecholamines in vertebrate tissues (Goodman and Gilman, 1970), interferes with

the induction of metamorphosis supports this hypothesis. As with all experiments in which substances are added to a sea water bath, effective dose and site of action are impossible to determine.

The three principal observations of this research are: 1) electrical stimulation of nerve centers induces metamorphosis, 2) stimulatory and inhibitory control is associated with the same regions of the larval nervous system, and 3) neurotransmitters can induce metamorphic responses in isolated tissues. These results support the proposal that the nervous system controls metamorphosis. Observations and experiments also indicate that control resides in the apical neuropile and the oral ganglion. It is tentatively suggested that a cue for metamorphosis from the adult environment is received by receptors that communicate with the larval nervous system. Nerve centers release inhibitory control and stimulate tissues to initiate the sequence of developmental events of metamorphosis. The mechanisms of inhibition and stimulation have not been determined. However, the nervous system has not been observed to make axonal connection with all the tissues that respond during metamorphosis, indicating that an alternative mechanism may be involved.

ACKNOWLEDGMENTS

Preliminary studies for the research reported here were conducted while I was a post-doctoral fellow in the laboratories of Dr. D. B. Bonar, University of Maryland, and Dr. M. E. Rice, Smithsonian Institution, Ft. Pierce Bureau. I am grateful to both of them for their support, encouragement, and advice. This research was supported by a Natural Sciences and Engineering Research Council of Canada University Research Fellowship and Operating Grant.

LITERATURE CITED

BURKE, R. D. 1978. The structure of the nervous system of the pluteus larva of *Strongylocentrotus purpuratus*. *Cell Tissue Res.* **191**: 233–247.

BURKE, R. D. 1980. Podial sensory receptors and the induction of metamorphosis in echinoids. *J. Exp. Mar. Biol. Ecol.* **47**: 223–234.

BURKE, R. D. 1982. Echinoid metamorphosis: retraction and resorption of larval tissues. Pp. 513–519 in *Proceedings of the International Echinoderms Conference, Tampa Bay, 1981*, J. M. Lawrence, ed. Balkema, Rotterdam.

CALDWELL, J. W. 1972. Development, metamorphosis, and substrate selection of the larvae of the sand dollar, *Mellita quinquesperforata* (Leske, 1778). M.Sc. Thesis, University of Florida, Gainsville, 63 pp.

CAMERON, R. A., AND R. T. HINEGARDNER. 1974. Initiation of metamorphosis in laboratory cultured sea urchins. *Biol. Bull.* **146**: 335–342.

CAMERON, R. A., AND R. T. HINEGARDNER. 1978. Early events in sea urchin metamorphosis, description and analysis. *J. Morphol.* **157**: 21–32.

CHIA, F. S. 1978. Perspectives: settlement and metamorphosis of marine invertebrate larvae. Pp. 283–285 in *Settlement and Metamorphosis of Marine Invertebrate Larvae*, F. S. Chia and M. E. Rice, eds. Elsevier, New York.

CHIA, F. S., AND R. D. BURKE. 1978. Echinoderm metamorphosis: fate of larval structures. Pp. 219–234 in *Settlement and Metamorphosis of Marine Invertebrate Larvae*, F. S. Chia and M. E. Rice, eds. Elsevier, New York.

CHIA, F. S., AND M. E. RICE, eds. 1978. *Settlement and Metamorphosis of Marine Invertebrate Larvae*. Elsevier, New York.

CLONEY, R. A. 1978. Ascidian metamorphosis: review and analysis. Pp. 255–282 in *Settlement and Metamorphosis of Marine Invertebrate Larvae*, F. S. Chia and M. E. Rice, eds. Elsevier, New York.

CLONEY, R. A. 1982. Ascidian larvae and the events of metamorphoses. *Am. Zool.* **22**: 817–826.

COBB, J. L. S. 1970. The significance of radial nerve cords in asteroids and echinoids. *Z. Zellforsh.* **108**: 457–474.

CRISP, D. J. 1974. Factors influencing settlement of marine invertebrate larvae. Pp. 177–265 in *Chemoreception in Marine Organisms*, P. T. Grant and A. M. Mackie, eds. Academic Press, London.

CRISP, D. J. 1976. Settlement responses of marine organisms. Pp. 83–124 in *Adaptations to Environment,* R. C. Newell, ed. Butterworths, London.

GOODMAN, L. S., AND A. GILMAN, eds. 1970. *The Pharmacological Basis of Therapeutics.* 4th ed. Macmillan, New York.

GRACE, A. S., AND B. S. BUNNY. 1980. Nigral neurons: intracellular recording and identification with L-dopa injection and histofluorescence. *Science* **210:** 654–656.

GUSTAFSON, T. 1969. Cell recognition and cell contacts during sea urchin development. Pp. 47–60 in *Cellular Recognition,* T. Smith and R. A. Good, eds. Appleton-Century-Crofts, New York.

HADFIELD, M. G. 1978. Metamorphosis in marine molluscan larvae: an analysis of stimulus and response. Pp. 165–176 in *Settlement and Metamorphosis of Marine Invertebrate Larvae,* F. S. Chia and M. E. Rice, eds. Elsevier, New York.

HIGHSMITH, R. C. 1982. Induced settlement and metamorphosis of sand dollar (*Dendraster excentricus*) larvae in predator-free sites: adult sand dollar beds. *Ecology* **63:** 329–337.

HINEGARDNER, R. T. 1969. Growth and development of the laboratory cultured sea urchin. *Biol. Bull.* **137:** 465–475.

KEENAN, L. C., AND H. KOOPWITZ. 1981. Limitations in identifying neurotransmitters within neurons by fluorescence histochemistry techniques. *Science* **214:** 1151–1152.

LITTLE, G. 1969. The larval development of the shrimp, *Palaemon macrodactylus* Rathbun, reared in the laboratory, and the effect of eyestalk expiration on development. *Crustaceana* **17:** 69–87.

MACBRIDE, E. W. 1903. The development of *Echinus esculentus* together with some points on the development of *E. miliaris* and *E. acutus. Phil. Trans. Roy. Soc. London B* **195:** 285–330.

MACBRIDE, E. W. 1914. The development of *Echinocardium cordatum,* Part 1. The external features of the development. *Q. J. Microsc. Sci.* **59:** 471–486.

MACBRIDE, E. W. 1918. The development of *Echinodardium cordatum,* Part 2. The development of the internal organs. *Q. J. Microsc. Sci.* **63:** 259–282.

MARSDEN, J. R., AND D. T. ANDERSON. 1981. Larval development and metamorphosis of the serpulid polychaete *Galeolaria caespitosa* Lamarck. *Aust. J. Mar. Freshwater Res.* **32:** 667–680.

MEADOWS, P. S. M., AND J. I. CAMPBELL. 1972. Habitat selection by aquatic invertebrates. *Adv. Mar. Biol.* **10:** 271–282.

MORSE, D. E., N. HOOKER, H. DUNCAN, AND L. JENSEN. 1979. γ-Aminobutyric acid, a neurotransmitter, induces planktonic abalone larvae to settle and begin metamorphosis. *Science* **204:** 407–410.

MORTENSEN, T. 1920. Notes on the development of the larval forms of some Scandinavian echinoderms. *Vidensk. Medd. Dansk. Naturhist. Foren.* **71:** 133–160.

PASSANO, L. M. 1961. The regulation of crustacean metamorphosis. *Am. Zool.* **1:** 85–95.

PENTREATH, V. W., AND J. L. S. COBB. 1972. Neurobiology of echinodermata. *Biol. Rev.* **47:** 363–392.

RYBERG, E. 1974. The localization of biogenic amines in the echinopluteus. *Acta. Zool.* **55:** 179–189.

RYBERG, E. 1977. The nervous system of the early echinopluteus. *Cell Tissue Res.* **179:** 157–167.

SCHELTEMA, R. S. 1974. Biological interactions determining larval settlement of marine invertebrates. *Thalassia Jugosl.* **10:** 263–296.

SHARPE, M. J., AND H. J. ATKINSON. 1980. Improved visualization of dopaminergic neurons in nematodes using the glyoxylic acid fluorescence method. *J. Zool. Lond.* **190:** 273–284.

SIMIONESCU, N., AND M. SIMIONESCU. 1976. Galloylglucoses of low molecular weight as mordant in electron microscopy. I. Procedure, and evidence for mordanting effect. *J. Cell Biol.* **70:** 608–621.

SMITH, J. E. 1965. Echinodermata. Pp. 1519–1558 in *Structure and function in the nervous systems of invertebrates. Vol. II,* T. H. Bullock and G. A. Horridge, eds. Freeman and Co., San Francisco.

STRATHMANN, M. 1968. *Methods in development series. I. General procedures and echinodermata-echinoidea.* Friday Harbor Laboratories, Friday Harbor.